AD NUMBER

AD864723

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; JAN 1970. Other requests shall be referred to the Army Biological Laboratory, Attn: Technical Release Branch [TID], Fort Detrick, MD 21701.

AUTHORITY

SMUFD, per d/a ltr

AD

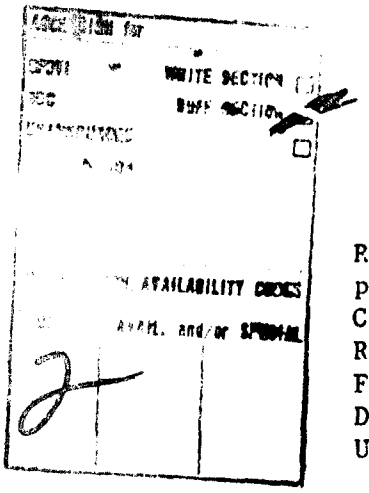
TECHNICAL MANUSCRIPT 579

IS PASTEURELLA TULARENSIS LIPOPOLYSACCHARIDE AN ENDOTOXIN?

Maurice L. Guss

JANUARY 1970

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland



DDC AVAILABILITY NOTICES

Qualified requesters may obtain copies of this publication from DDC.

Foreign announcement and dissemination of this publication by DDC is not authorized.

Release or announcement to the public is not authorized.

DISPOSITION INSTRUCTIONS

Destroy this publication when it is no longer needed. Do not return it to the originator.

The findings in this publication are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701

TECHNICAL MANUSCRIPT 579

IS *PASTEURELLA TULARENSIS* LIPOPOLYSACCHARIDE AN ENDOTOXIN?

Maurice L. Guss

Medical Bacteriology Division
BIOLOGICAL SCIENCES LABORATORIES

Project 1B562602AD01

January 1970

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ABSTRACT

Pasteurella tularensis cell wall or lipopolysaccharide (LPS), unlike *Salmonella enteritidis* LPS, is nontoxic for mice previously sensitized by enterotoxin B. Studies in normal mice challenged with *P. tularensis* 425, a strain of intermediate virulence, demonstrated that *P. tularensis* LPS, like *S. enteriditis* LPS, can increase host resistance with resultant reduction in disease severity and mortality. In contrast to *S. enteritidis* LPS, *P. tularensis* LPS administration to tularemia-immunized mice 24 hours after challenge with highly virulent *P. tularensis* SCHU S4 resulted in little or no reduction in resistance. The LPS of each species enhanced resistance of nonimmunized mice to SCHU S4 challenge when LPS was inoculated 24 or 72 hours before infection. However, in immunized mice, the enhancement of host resistance was significant only when mice were treated with *S. enteritidis* LPS before infection. Therefore, *P. tularensis* LPS as an endotoxin is unusual among the endotoxins of gram-negative bacteria, and further study of methods of extraction and structural identification may lead to a better understanding of the origins of the varied activities within this complex molecule and of LPS endotoxins in general.

IS PASTEURELLA TULARENSIS LIPOPOLYSACCHARIDE AN ENDOTOXIN?*

Whether classic endotoxin is elaborated by Pasteurella tularensis has not yet been proved. Indirect evidence for the presence of endotoxin via the phenomenon of acquired tolerance was reported by Greisman et al.[1] Stefanye[2] noted that P. tularensis lipopolysaccharide (LPS) prepared by phenol or ethylene glycol extraction was antigenic and dermonecrotic, but not toxic, pyrogenic, or able to reduce nonspecific host resistance. In a study of the immunogenic potency of purified cell walls of P. tularensis, I observed[3] that the cell wall fraction in the monkey was immunogenic, antigenic, slightly dermonecrotic, but not toxic or pyrogenic.

The current report concerns the toxicity of P. tularensis LPS and cell wall and the functional relationship of P. tularensis LPS to nonspecific resistance.

The studies were carried out in male, albino, Swiss mice weighing 18 to 22 g. Data are evaluated on the basis of the mouse protection index (MPI) described by Meyer and Foster.[4] The MPI includes in one statistic both percentage mortality and length of survival. Salmonella enteritidis LPS (Difco, Westphal) and P. tularensis LPS were prepared in 0.9% saline and heated at 80 C for 10 minutes before use. Enterotoxin B was also prepared in 0.9% saline. Pasteurella tularensis cell wall material was obtained by sonication of washed cells and was purified by repeated washing and differential centrifugation. The P. tularensis strains used for challenge were highly virulent SCHU S4 or 425 of intermediate virulence. Pasteurella tularensis LPS, kindly supplied by Dr. D. Stefanye, was extracted by the Westphal phenol procedure.

Because Sugiyama et al.[5] showed quantitative correlation of enterotoxin B as an agent for sensitizing mice to the action of LPS from several different gram-negative bacteria, it became a matter of interest whether mice could be sensitized by enterotoxin B to P. tularensis cell walls or LPS. In the experiment reported in Table 1, 12 to 15 mice were used per group. Intraperitoneal (IP) inoculation of 4.5 or 45 μg enterotoxin B was followed 4 hours later by IP injection of 125, 250, or 500 μg P. tularensis cell wall or LPS or 250 μg S. enteritidis LPS. The treated animals were held for 72 hours. No mortality occurred with either the P. tularensis cell wall or LPS following enterotoxin sensitization but the synergism was quite marked when S. enteritidis LPS was used. This demonstrates another noncharacteristic endotoxin property of P. tularensis LPS, in that it fails to create a physiological response identified with endotoxin.

---

* This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the author to ascertain when and where it may appear in citable form.

TABLE 1. EFFECT OF SENSITIZATION OF MICE WITH ENTEROTOXIN B ON TOXICITY OF PASTEURELLA TULARENSIS CELL WALL MATERIAL OR LIPOPOLYSACCHARIDE

Enterotoxin B, μg/mouse	Test Material, μg/mouse	Mortality, %
4.5 or 45	None	0
0	P. tularensis cell wall, 125, 250, or 500	0
4.5 or 45	P. tularensis cell wall, 125, 250, or 500	0
0	P. tularensis LPS, 125, 250, or 500	0
4.5 or 45	P. tularensis LPS, 125, 250, or 500	0
0	S. enteritidis LPS, 250	0
4.5	S. enteritidis LPS, 250	50
45	S. enteritidis LPS, 250	95

The rest of this paper is concerned with the alteration of host resistance by S. enteritidis LPS and by P. tularensis LPS. Assay of effect was made by challenging treated normal or immunized mice with P. tularensis SCHU S4, or 425. Mice were vaccinated subcutaneously (SC) with 50 or 500 viable cells of the live tularemia vaccine strain LVS 4 or 7 weeks before study. All experiments were terminated at 21 days after bacterial cell challenge.

Table 2 shows the effect of S. enteritidis LPS in normal mice challenged IP with approximately two cells of strain 425. S. enteritidis LPS given IP 24 hours before to 72 hours after challenge enhanced host resistance, with the maximal effectiveness noted at treatment 72 hours before infection. Data from a similar experiment are recorded in Table 3, but this time P. tularensis LPS was investigated. Here we see not only the reduction of the MPI but also the nontoxicity of the P. tularensis LPS. Thus, with regard to alteration of host resistance, P. tularensis LPS shares with S. enteritidis LPS the property of reducing host susceptibility to infection with attenuated strain 425.

Data from a similar experiment are given in Table 4, except that all inoculations were SC, 100 cells of the highly virulent strain SCHU S4 were used for challenge, and groups of vaccinated mice are included. Protection afforded by the vaccine is evident by reduction of mortality and the increased time to death from tularemia. However, when S. enteritidis LPS was administered 24 hours after challenge, the protective effect of the vaccine was nullified so that the treated immunized mice reacted to challenge, superficially at least, exactly as did normal challenged mice. When S. enteritidis LPS was given 24 or 72 hours prior to challenge, the resistance of both the normal and vaccinated animals was increased.

TABLE 2. EFFECT OF *SALMONELLA ENTERITIDIS* LIPOPOLYSACCHARIDE ON THE RESISTANCE OF THE MOUSE TO INFECTION WITH *PASTEURELLA TULARENSIS* 425

Treatment	Mortality No. Dead/Total	Mortality %	Avg Day of Death	Mouse Protection Index[a]
P. tularensis 425, approx two cells	45/47	96	6.6	14.5
S. enteritidis LPS, 200 µg	39/192	20	18.1	1.1
425 — 24 hr — LPS	8/11	73	12.0	6.1
425 — 0 hr — LPS	24/43	56	15.2	3.7
LPS — 24 hr — 425	23/35	66	13.7	4.8
LPS — 48 hr — 425	4/11	36	16.3	2.2
LPS — 72 hr — 425	0/12	0	(21.0)[b]	-

a. Mouse protection index = $\frac{\text{mortality, \%}}{\text{average day of death}}$.

b. Experiment terminated 21 days after bacterial challenge.

TABLE 3. EFFECT OF *PASTEURELLA TULARENSIS* LIPOPOLYSACCHARIDE ON THE RESISTANCE OF THE MOUSE TO INFECTION WITH *PASTEURELLA TULARENSIS* 425

Treatment	Mortality No. Dead/Total	Mortality %	Avg Day of Death	Mouse Protection Index[a]
P. tularensis 425, approx two cells	45/47	96	6.6	14.5
P. tularensis LPS, 200 µg	0/96	0	(21.0)[b]	-
425 — 0 hr — LPS	19/32	59	12.7	4.6
LPS — 24 hr — 425	19/32	59	12.8	4.6

a. Mouse protection index = $\frac{\text{mortality, \%}}{\text{average day of death}}$.

b. Experiment terminated 21 days after bacterial challenge.

TABLE 4. EFFECT OF SALMONELLA ENTERITIDIS LIPOPOLYSACCHARIDE ON THE RESISTANCE OF NORMAL AND IMMUNIZED MICE TO INFECTION WITH PASTEURELLA TULARENSIS SCHU S4

Treatment	Normal Mice				Vaccinated Mice[a]			
	Mortality		ADD[b]	MPI[c]	Mortality		ADD[b]	MPI[c]
	No. Dead/Total	%			No. Dead/Total	%		
P. tularensis SCHU S4, 100 cells	16/16	100	4.6	21.7	12/16	75	9.9	7.6
SCHU S4 — 24 hr — LPS[d]	16/16	100	3.9	25.6	15/16	94	4.5	20.9
LPS — 24 hr — SCHU S4	16/16	100	6.4	15.6	10/16	63	13.8	4.6
LPS — 72 hr — SCHU S4	16/16	100	6.7	14.9	5/16	31	16.0	1.9

a. 50 LVS organisms SC.

b. ADD = average day of death.

c. MPI = mouse protection index = $\frac{\text{mortality, \%}}{\text{average day of death}}$.

d. S. enteritidis LPS 200 µg.

Table 5 contains the data of a companion experiment using *P. tularensis* LPS. In contrast to the effect of *S. enteritidis* LPS given 24 hours after challenge, the *P. tularensis* LPS had little or no effect on reduction of the resistance status of the immunized mice. However, host resistance increased among the normal mice when *P. tularensis* LPS was inoculated 24 and 72 hours before infection.

The information presented here indicates that the LPS of *P. tularensis* may be quite different structurally from LPS of other gram-negative bacteria. It is nontoxic for mice, has no apparent synergistic interaction with enterotoxin, and is less effective than standard LPS in alteration of host response. *Pasteurella tularensis* has what may be an unusual LPS, which, based on present information, should not yet be referred to as endotoxin. It may be considered an incomplete endotoxin; additional study of methods of isolation and structural identification may lead to a better understanding of the origins of the varied activities within this complex molecule and of endotoxin behavior in general.

TABLE 5. EFFECT OF *PASTEURELLA TULARENSIS* LIPOPOLYSACCHARIDE ON THE RESISTANCE OF NORMAL AND IMMUNIZED MICE TO INFECTION WITH *PASTEURELLA TULARENSIS* SCHU S4

Treatment	Normal Mice				Vaccinated Mice[a]			
	Mortality		ADD[b]	MPI[c]	Mortality		ADD[b]	MPI[c]
	No. Dead/Total	%			No. Dead/Total	%		
P. tularensis SCHU S4, 100 cells	16/16	100	4.9	20.4	1/16	6	20.3	0.30
SCHU S4 — 24 hr — LPS[d]	16/16	100	4.9	20.4	2/16	13	19.1	0.68
LPS — 24 hr — SCHU S4	16/16	100	5.8	17.2	1/16	6	20.1	0.30
LPS — 72 hr — SCHU S4	16/16	100	6.4	15.6	0/16	0	(21.0)[e]	-

a. 500 LVS organisms SC.
b. ADD = average day of death.
c. MPI = mouse protection index = $\frac{\text{mortality, \%}}{\text{average day of death}}$
d. *P. tularensis* LPS 200 μg.
e. Experiment terminated 21 days after bacterial challenge.

LITERATURE CITED

1. Greisman, S.D.; Hornick, R.B.; Carozza, F.A., Jr.; Woodward, T.E. 1963. The role of endotoxin during typhoid fever and tularemia in man: I. Acquisition of tolerance to endotoxin. J. Clin. Invest. 42:1064-1075.

2. Stefanye, D. 1961. Lipopolysaccharides of Pasteurella tularensis. Bacteriol. Proc. 1961:129.

3. Guss, M.L. 1965. Immunogenic potency of Pasteurella tularensis cell walls. Bacteriol. Proc. 1965:51.

4. Meyer, K.F.; Foster, L.E. 1948. Measurement of protective serum antibodies in human volunteers inoculated with plague prophylactics. Stanford Med. Bull. 6:75-79.

5. Sugiyama, H.; McKissic, E.M.; Bergdoll, M.S.; Heller, B. 1964. Enhancement of bacterial endotoxin lethality by staphylococcal enterotoxin. J. Infect. Dis. 114:111-118.

Unclassified
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)*

Department of the Army
Fort Detrick, Frederick, Maryland, 21701

2a. REPORT SECURITY CLASSIFICATION

Unclassified

2b. GROUP

3. REPORT TITLE

IS PASTEURELLA TULARENSIS LIPOPOLYSACCHARIDE AN ENDOTOXIN?

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

5. AUTHOR(S) *(First name, middle initial, last name)*

Maurice L. Guss

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
January 1970	13	5

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. 1B562602AD01	Technical Manuscript 579
c. Task-Work Unit 01-020	9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
d. DD 1498 Agency Accession DA OL 0235	CMs 6639 SMUFD-AE-T 49551

10. DISTRIBUTION STATEMENT

Qualified requesters may obtain copies of this publication from DDC.
Foreign announcement and dissemination of this publication by DDC is not authorized.
Release or announcement to the public is not authorized.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	Department of the Army Fort Detrick, Frederick, Maryland, 21701

13. ABSTRACT

Pasteurella tularensis cell wall or lipopolysaccharide (LPS), unlike Salmonella enteritidis LPS, is nontoxic for mice previously sensitized by enterotoxin B. Studies in normal mice challenged with P. tularensis 425, a strain of intermediate virulence, demonstrated that P. tularensis LPS, like S. enteritidis LPS, can increase host resistance with resultant reduction in disease severity and mortality. In contrast to S. enteritidis LPS, P. tularensis LPS administration to tularemia-immunized mice 24 hours after challenge with higly virulent P. tularensis SCHU S4 resulted in little or no reduction in resistance. The LPS of each species enhanced resistance of nonimmunized mice to SCHU S4 challenge when LPS was inoculated 24 or 72 hours before infection. However, in immunized mice, the enhancement of host resistance was significant only when mice were treated with S. enteritidis LPS before infection. Therefore, P. tularensis LPS as an endotoxin is unusual among the endotoxins of gram-negative bacteria, and further study of methods of extraction and structural identification may lead to a better understanding of the origins of the varied activities within this complex molecule and of LPS endotoxins in general.

14. Key Words

Pasteurella tularensis
Salmonella enteritidis
Lipopolysaccharide
Host resistance
Endotoxin

DD FORM 1 NOV 68 1473 REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

Unclassified
Security Classification